be constructed by applying a labeled analyte on the surface of the detection working electrode **42**, the signal provided by the detection working electrode **42** is inversely proportional to the analyte concentration in the test sample. It should be understood that the potential may be applied either before or after the sample has been placed in the detection area (e.g. electrodes). The potential is preferably applied after the sample has reached the detection area to prevent continued electrochemical process during the formation of immunocomplex on the electrode surface. The formation time may be from about 1 second to about 15 minutes, depending on the sample size, channel, size, membrane size, and/or electrode size.

[**0061**] Due to the use of separate substrates, the present invention may also easily accommodate more than one analyte. For example, in one embodiment, the auxiliary electrode on the second substrate **80** may be an additional detection working electrode. The detection working electrodes on the substrates **40** and **80** may be used to detection different analytes, and may consequently be treated with different affinity reagents. The counter/reference electrode(s) for each detection working electrode may be present on the substrates **40** and/or **80**, or may remain altogether separate, such as being disposed on an additional substrate.

[**0062**] Various parameters of the detection technique may be utilized to improve the consistency and accuracy of the assay device. For example, variations of fabrication processes, such as electrode coating, flow control, sample size, mediator efficiency, etc, may have an impact on data collection. Thus, in one embodiment, the time at which current readings are measured may be selected to achieve improved results. Specifically, when a potential is applied, the initial reading of the current may be inaccurate or less reliable. Accordingly, the time at which the current reading is first recorded may be after applying the potential. Thus, in some embodiments, the first recording is from about 0.001 seconds to about 10 minutes, in some embodiments from about 0.1 seconds to about 1 minute, in some embodiments from about 0.5 to about 20 seconds, and in some embodiments, from about 1 to about 10 seconds, after applying the potential. In addition, the current readings may also be recorded in flexible time intervals. If desired, for example, the number of readings taken at the beginning of the recordings may be greater than the number taken at the end. This is due primarily to the fact that, at the later stages of the recordings, the decrease in measured current is usually more profound than the magnitude of the potential pulse.

[**0063**] Regardless of the detection environment, the total charge is normally the same for a given analyte concentration because the current measurements are obtained at intervals over the course of the entire assay and integrated over time to obtain the total amount of charge, Q, passed to or from the electrode. Q is then used to calculate the concentration of the analyte. For instance, the total charge, Q, may be directly calculated when the redox label is able to generate a detection signal. The completion of the electrochemical reaction is signaled when the current reaches a steady-state value that indicates all or nearly all of the redox labels on the electrode surface have been electrolyzed. In such cases, at least 90%, in some embodiments at least 95%, and in some embodiments, at least 99% of the complexes are electrolyzed. In other cases, however, the redox label may not be able to generate a measurable detection signal without amplification. For instance, an enzyme label may require a substrate to provide amplification of the detection current. If desired, the substrate may be used in excess to ensure that the detection signal reaches the a measurable level. In some embodiments, for example, the ratio of the substrate to the complexes formed on the electrode surface is at least 10:1, in some embodiments at least 100:1, in some embodiments at least 1,000:1, and in some embodiments, at least 10,000:1.

[**0064**] Although various embodiments of assay formats and devices have been described above, it should be understood, that the present invention may utilize any assay format or device desired, and need not contain all of the components described above. Further, other well-known components of assay formats or devices not specifically referred to herein may also be utilized in the present invention. For example, various assay formats and/or devices are described in U.S. Pat. No. 5,508,171 to Walling, et al.; U.S. Pat. No. 5,534,132 to Vreeke. et al.; U.S. Pat. No. 6,241,863 to Monboucuette; U.S. Pat. No. 6,270,637 to Crismore, et al.; U.S. Pat. No. 6,281,006 to Heller, et al.; and U.S. Pat. No. 6,461,496 to Feldman, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

[**0065**] In addition, it should be understood that both sandwich and competitive assay formats may be formed according to the present invention. Techniques and configurations of sandwich and competitive assay formats are well known to those skilled in the art. For instance, sandwich assay formats typically involve mixing the test sample with labeled antibodies so that complexes of the analyte and the labeled antibody are formed. These labeled complexes contact a detection zone where they bind to another antibody and become immobilized, thereby indicating the presence of the analyte. Some examples of such sandwich-type assays are described by U.S. Pat. No. 4,168,146 to Grubb, et al. and U.S. Pat. No. 4,366,241 to Tom, et al., which are incorporated herein in their entirety by reference thereto for all purposes. In a competitive assay, a labeled analyte or analyte-analog competes with an unlabeled analyte in the test sample for binding to an antibody immobilized at the detection zone. Competitive assays are typically used for detection of analytes such as haptens, each hapten being monovalent and capable of binding only one antibody molecule. Examples of competitive immunoassay devices are described in U.S. Pat. No. 4,235,601 to Deutsch, et al., U.S. Pat. No. 4,442,204 to Liofta, and U.S. Pat. No. 5,208,535 to Buechler, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

[**0066**] The present invention provides a low-cost, flow-through assay device that may provide accurate analyte detection. The assay devices of the present invention may be produced as a single test for detecting an analyte or it may be formatted as a multiple test device. The uses for the assay devices of the present invention include, but are not limited to, detection of chemical or biological contamination in garments, such as diapers, the detection of contamination by microorganisms in prepacked foods such as fruit juices or other beverages, and the use of the assay devices of the present invention in health diagnostic applications such as diagnostic kits for the detection of antigens, microorganisms, and blood constituents. It should be appreciated that the present invention is not limited to any particular use or application.